HALOPHILIC ESTERASE PURIFICATION FROM *HALOARCULA MARISMORTUI*.

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Introduction. Archaea were formally proposed as the third domain of life only 22 years ago. Archaea have traditionally been grouped into methanogens, thermoacidophiles and halophiles, which are often classified as extremophiles [1]. Halophiles are extremophiles that thrive in environments with very high concentrations of salt [2] and their enzymes are also stable and active in solvents with low water activity, and consequently they are considered as robust biocatalysts with potential applications in synthesis using non-aqueous media. Lipolytic enzymes (lipases and esterases) catalyze both the hydrolysis and synthesis of ester compounds [3]. These enzymes are the most important, from the viewpoint of the range of biotechnological applications that they are able to perform. Esterases and lipases have been detected in *Haloarcula marismortui* (a halophilic archeon) [2,3,4]. These esterases were cloned, expressed in *E. coli* and biochemically characterized [2,4]. The aim of this work was the partial purification and characterization of a novel lipolytic enzyme from *H. marismortui*

Methods. *Haloarcula marismortui* (3752) was obtained from DSMZ and cultured in ATCC 2185 medium. The cell culture was incubated at 37°C and 175 rpm for 48h; in 1L Erlenmeyer baffled flasks until 2.5 g/L OD $_{600nm}$ of 3.3 was reached. Esterase and lipase activities were spectrophotometrically assayed [5], by measuring the hydrolysis rate of *p*-nitrophenyl valerate (pNPV) for esterase and *p*-nitrophenyl laurate (pNPL) for lipase. 8L Culture broth was centrifuged at 4°C, 4,500 rpm for 1h, and the cell pellet (20g) was resuspended in 2L solution containing 20mM Tris-HCl and 0.15M NaCl, stored at -20°C for 12 h and sonicated to guarantee a complete disruption. Protein was determined by Lowry method [6]. Intracellular crude extract (ICE) was centrifuged at 10,000 rpm for 1h to remove cell debris and filtered with 0.45μm cellulose membranes. Lipolytic enzyme was purified in three chromatographic steps: (A) butyl sepharose™ 4FF column (110 ml), (B) butyl sepharose™ 4FF column (60 ml) and (C) Superdex™ G200 (16/60). Finally, an electroelution (D) was made. Each step of purification was monitored by electrophoresis: native gels, 4-Methylumbelliferyl butyrate (MUFB) zymograms [7] and SDS-PAGE.

Results. A partial purification of an esterase was achieved (Table 1). At least three lipolytic enzymes were detected by MUFB zimograms, one of them was purified and its molecular weight estimated between 45-50 kDa by SDS-PAGE (Fig 1). The enzymes pool from the first chromatography (A) was assayed on different substrates: butyric monoglyceride, butyric diglyceride, vinyl butyrate, and vinyl laurate; obtaining the following enzyme activities: 11, 10.7, 42 and 16 U/g, respectively.

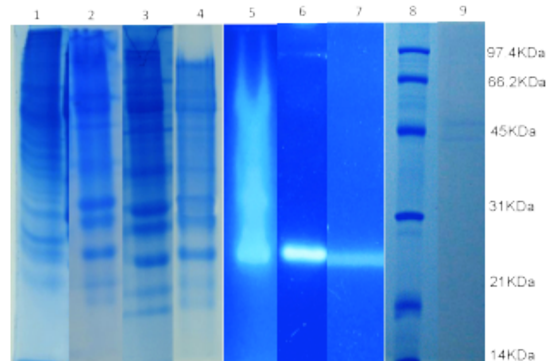

Fig.1 Lane 1, 2, 3 and 4 are native gels; 5, 6 and 7 are MUFB zymograms; 8 and9 are SDS-PAGE. Enzymes pools were applied in each lane: 1 from ICE, 2 &5 fromA, 3 & 6 from B, 4 & 7 from C, 8 molecular weight markers and 9 from D.

Table 1. Purification of an esterase from *Haloarcula marismortui*.

Steps	Activity (U)	Protein (g)	Specific activity (U/g)	Purification fold	Yield %
ICE	7.16	5.9	1.2	1	100
A	2.84	1.80E-01	15.8	13.2	40
B	2.07	6.00E-02	34.5	28.8	29
C	0.56	2.60E-03	215.4	179.5	8
D	0.10	3.20E-05	2937.5	2447.9	1.3

Conclusions. Halophilic lipolytic enzymes were observed for first time using MUFB zymograms. From the ICE, several enzymes with lipolytic activities were detected. One of them, was purified (an esterase), reaching a specific activity of 2.94 U/mg (in pNPV), while the recombinant esterase LipC had a specific activity of 0.86 U/mg (in pNP-Acetate) [2].

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